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The synthesis and characterisation of 2-*O*-(6-*O*-L-*glycero*-α,β-D-*manno*-heptopyranosyl-α-D-glucopyranosyl)-α,β-D-glucopyranose *

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ABSTRACT

The title compounds were synthesised, and appropriate derivatives were characterised by GLC, GLC–MS, and NMR spectroscopy. The GLC and GLC–MS data proved 2-*O*-(6-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-α-D-glucopyranosyl)-D-glucopyranose to be a constituent of the outer-core region of the lipopolysaccharide from *Escherichia coli* K-12, indicating the heptosyl residue to be linked to the terminal glucopyranose residue.

INTRODUCTION

Five types of core (R1–R4 and K-12) of the lipopolysaccharides (LPS) of *Escherichia coli* have been described [1], of which the structures of the hexose region (outer core) have been reported [2,3] and that of the K-12 core has been revised [4]. Only fragmentary work has been reported on the structures of the heptose–3-deoxy-D-*manno*-octulosonic acid (Kdo)-region (inner core) from the LPS of the R1, R2, and R4 cores, whereas the structures of the inner-core regions from the LPS of the R3 and K-12 cores have been elucidated [3,4]. For the K-12 core, it was shown that, in addition to 3-*O*-(7-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-L-*glycero*-α-D-*manno*-heptopyranosyl)-L-*glycero*-D-*manno*-heptopyranose, a characteristic constituent [1] of the inner-core region of many LPS, one L-*glycero*-D-*manno*-

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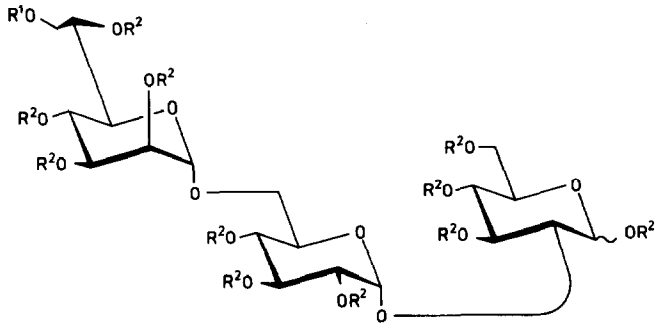
heptopyranosyl residue was linked to the outer core. 6-*O*-L-*glycero*-α-D-*manno*-Heptopyranosyl-D-glucopyranose was isolated [4] from the LPS and its identity was proved by chemical synthesis [5]. However, the position of the heptose in the outer-core region, which contains three glucosyl residues [4], was not determined. It was possible to identify [4] reduced and methylated L,D-Hep*p*-(1 → 6)-D-Glc*p*-(1 → 2)-D-Glc*p* by GLC–MS and methylation analysis, but the low yield did not allow detailed NMR analysis. For this purpose, and in order to prove the structure of the trisaccharide, the synthesis of the title compounds and their characterisation by GLC, GLC–MS, and NMR spectroscopy was undertaken.

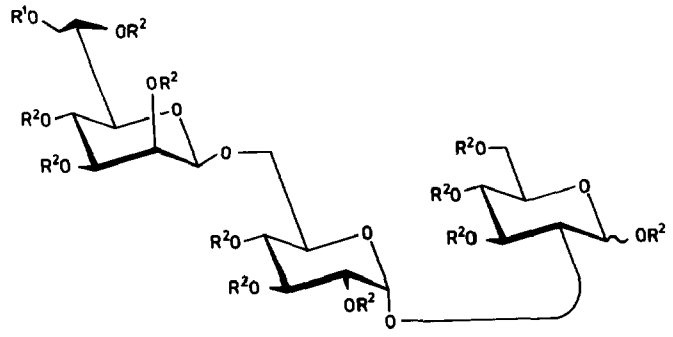
RESULTS AND DISCUSSION

Synthesis of 2-O-(6-O-L-glycero-α- *and* -β-D-manno-*heptopyranosyl*-α-D-*glucopyranosyl*)-α,β-D-*glucopyranose* (**1** *and* **2**).—In order to obtain **1** and **2**, benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-β-D-glucopyranoside (**12**) was condensed with 7-*O*-allyl-2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate [6] (**24**). Compound **12** was synthesised from benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside [7–9] (**16**), obtained (70%) by Ogawa and Takahashi [9] by trimethylsilyl triflate-catalysed rearrangement of 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-benzyloxyethylidene)-α-D-glucopyranose (**14**), followed by deacetylation. Essentially the same reaction sequence was used with benzylation of 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-benzyloxyethylidene)-α-D-glucopyranose (**13**) to give **14**, the orthoester ring of which was opened with benzyl alcohol to yield **15**, and *O*-deacetylation then gave **16**. Slight modifications of the reaction conditions increased the yield of **16** to 80%.

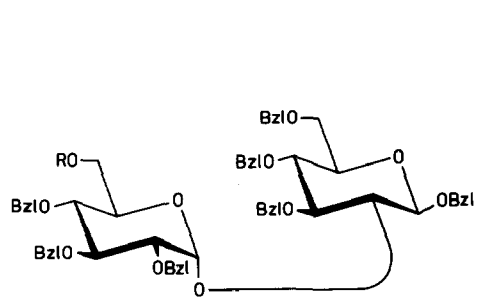
6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**19**), obtained from 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose [10] (**17**) by acetolysis (→ **18**) and



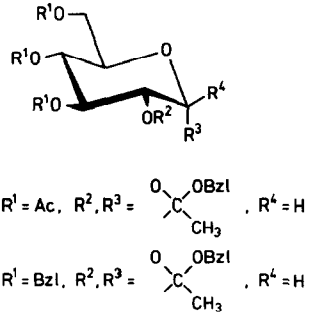
1 R^1, R^2 = H
7 R^1 = All, R^2 = Bzl
9 R^1 = H, R^2 = Bzl



2 $R^1, R^2 = H$
8 $R^1 = All, R^2 = Bzl$
10 $R^1 = H, R^2 = Bzl$

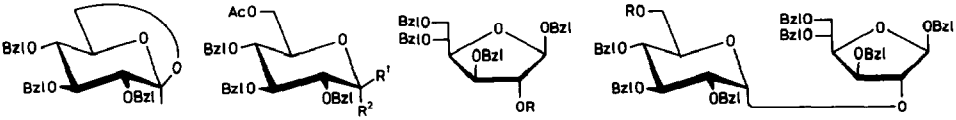


11 R = Ac
12 R = H



13 $R^1 = Ac, R^2, R^3 =$ $, R^4 = H$
14 $R^1 = Bzl, R^2, R^3 =$ $, R^4 = H$
15 $R^1 = Bzl, R^2 = Ac, R^3 = H, R^4 = OBzl$
16 $R^1 = Bzl, R^2 = H, R^3 = H, R^4 = OBzl$

then reaction [11] with $TiBr_4$, was condensed with **16** in the presence of $Hg(CN)_2$ to give the disaccharide derivative **11** as the sole product. Deacetylation of **11** then furnished **12** (43%).



17

18 $R^1, R^2 = H, OAc$
19 $R^1 = H, R^2 = Br$

20 R = Ac
21 R = H

22 R = Ac
23 R = H

An alternative to **12** was the furanose analogue **23**. Thus, benzyl 3,5,6-tri-*O*-benzyl-β-D-glucofuranoside (**21**), prepared by reaction of 1,2-di-*O*-acetyl-3,5,6-tri-*O*-benzyl-α,β-D-glucofuranose [12] with benzyl alcohol in the presence of trimethylsilyl triflate to give the β-glycoside **20** and then deacetylation, was condensed with **19** to give the disaccharide derivative **22** as the sole product (53%). Deacetylation of **22** gave **23**. However, **23** did not possess any particular advantage over **12**.

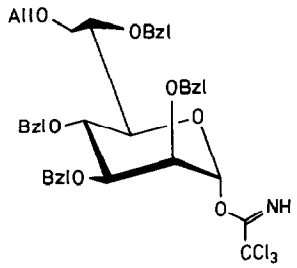
TABLE I

Retention times [a] (T) of derivatives in GLC

Compound	T	Compound	T
3	1.68/1.70	**5**	1.20 [b]
4	1.74/1.78	**6**	1.23 [b]
25	1.68	**27**	1.14
26	1.70	**28**	1.17

[a] Relative to maltitol nona-acetate, T 1.00. [b] Anomers not separated.

When **12** and **24** were reacted in the presence of anhydrous toluene-*p*-sulfonic acid, 60.6% of a 1:1.3 α,β-mixture of the trisaccharide derivatives **7** and **8** was obtained, which was resolved by chromatography. Deallylation of **7** and **8** gave **9** and **10**, respectively, debenzylation of which gave **1** and **2**, respectively.



24

Characterisation of acetylated and methylated derivatives of **1** *and* **2**.—Acetylation of **1** and **2** afforded the α,β-mixtures **3** and **4**, and the four products could be separated by GLC (Table I). The EIMS data are listed in Table II and the common fragmentation pattern is shown in Fig. 1. Most of the peaks were derived from the ions at m/z 403 and 331 (cleavage of the glycosidic bonds) by loss of ketene (−42) and acetic acid (−60). The ion at m/z 241 was the base peak. The intensities of the ions at m/z 139, 169, and 109 were significantly higher in the spectra of the anomers of the β-linked derivative **4**.

The alditol acetate derivatives (**25** and **26**) obtained from **1** and **2** could also be separated by GLC (Table I). The EIMS data are shown in Table II and the common fragmentation pattern in Fig. 1. The ions at m/z 403 and 376 (cleavage of the glycosidic bonds) and their secondary ions obtained by loss of ketene and acetic acid represented the majority of fragments. The ions at m/z 241 and 139 were the base peaks in the spectra of **25** and **26**, respectively. The ions at m/z 223 and 283 were missing in the spectrum of **25**, and those at m/z 172, 214, and 343 from the spectrum of **26**. In general, the fragment intensities were higher in the spectrum of **26**.

The methylated compounds **5** and **6** were identified in GLC by their relative retention times (Table I), but the respective pairs of anomers were not resolved.

TABLE II

EIMS data of **3**, **4**, **25**, and **26**

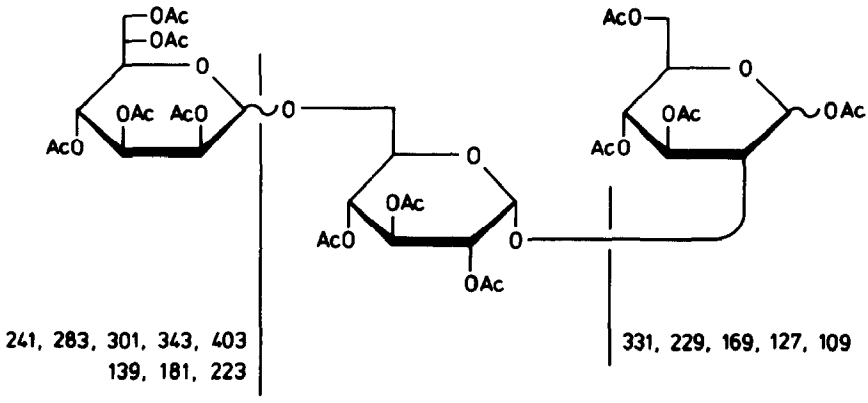
Compound	*m/z* (% of base peak)
3	81 (21.8/22.5), 85 (12.1/10.8), 97 (38.7/47.3), 103 (17.8/18.4), 109 (42.9/51.9), 110 (19.4/21.3), 111 (17.5/18.4), 115 (21.3/22.3), 127 (20.3/25.4), 139 (71.0/82.0), 145 (14.1/15.9), 152 (15.7/15.8), 153 (20.1/18.8), 155 (25.2/28.4), 163 (1.3/–), 169 (35.2/37.6), 181 (27.6/28.4), 223 (4.0/4.6), 229 (6.2/6.5), 241 (100.0/100.0), 283 (3.6/4.5), 301 (4.3/4.9), 331 (2.8/3.0), 343 (1.0/–), 403 (48.6/55.7)
4	81 (43.9/42.2), 85 (19.5/15.2), 97 (65.0/60.3), 103 (28.1/32.7), 109 (87.7/65.5), 110 (36.3/27.7), 111 (34.4/31.2), 115 (40.1/41.5), 127 (39.7/36.6), 139 (94.2/94.9), 145 (25.8/15.7), 152 (29.2/23.9), 153 (43.4/49.2), 155 (48.9/40.5), 157 (24.2/19.2), 169 (63.1/52.5), 181 (29.9/32.8), 223 (4.9/5.1), 229 (14.8/7.6), 241 (100.0/100.0), 283 (5.0/–), 301 (5.4/5.1), 331 (–/5.4), 343 (–/1.6), 403 (43.1/41.5)
25	81 (22.1), 85 (18.3), 97 (34.5), 103 (37.8), 109 (36.1), 110 (18.7), 111 (25.7), 112 (27.2), 115 (39.6), 127 (20.6), 139 (81.4), 140 (20.3), 145 (22.0), 153 (30.1), 154 (47.4), 155 (31.5), 157 (20.7), 169 (21.6), 172 (4.4), 181 (28.9), 214 (4.8), 217 (4.7), 229 (3.5), 241 (100.0), 274 (10.8), 301 (5.2), 343 (1.2), 376 (64.8), 403 (61.1)
26	81 (47.1), 85 (32.0), 97 (51.2), 103 (64.0), 109 (78.6), 110 (37.2), 111 (44.6), 112 (52.6), 115 (59.4), 127 (35.2), 139 (100.0), 140 (24.9), 145 (31.9), 153 (56.6), 154 (67.0), 155 (53.7), 157 (37.0), 169 (43.3), 181 (33.3), 217 (10.2), 223 (4.1), 229 (7.4), 241 (96.6), 274 (15.2), 283 (6.8), 301 (7.7), 376 (65.3), 403 (45.1)

The EIMS data are given in Table III. The mol wt of 702 was determined by CI(ammonia)-MS [*m/z* 720, $(M + 18)^+$]. Compared to the acetylated derivatives **3** and **4**, the identical fragmentation of **5** and **6** was rather poor (Fig. 2) and the ion intensities were similar, so that the compounds could not be differentiated. The majority of the ions were derived from cleavages of the glycosidic bonds, e.g., at *m/z* 219, 263, and 423, and their secondary ions obtained by loss of methanol (–32). Two J_1 fragments [13] were found at *m/z* 279 and 483.

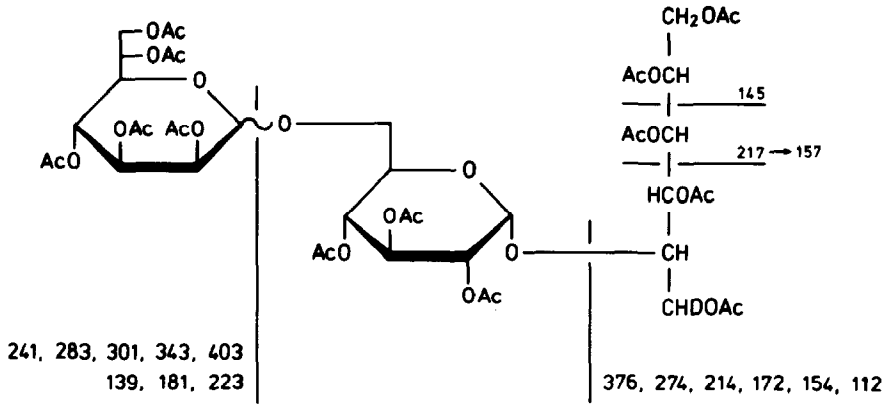
The reduced and methylated derivatives **27** and **28** were separated by GLC (Table I). Table III shows the EIMS data. The mol wt was determined by CI(ammonia)-MS. [*m/z* 737, $(M + 18)^+$]. The ions at *m/z* 199 and 236 were significantly more intense in the spectrum of **27** and those at *m/z* 408 and 440 were missing from the spectrum of **28**. The fragmentation patterns (Fig. 2) were similar. Most of the fragments were obtained from cleavage of the glycosidic bonds (ions at *m/z* 236 and 263) and their secondary ions by loss of methanol. Some fragmentation occurred in the alditol chain (ions at *m/z* 133 and 177, and the secondary ions of the latter at *m/z* 113 and 145). Two J_1 fragments [13] were found, at *m/z* 296 and 500.

The ^{1}H- and ^{13}C-NMR data for **3** and **4** are given in Tables IV and V.

The GLC and GLC–MS data [4] of the methylated Hep–Glc–Glcol derived from the LPS of *E. coli* K-12 were identical to those for **27**; hence, the structure 2-*O*-(6-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-α-D-glucopyranosyl)-D-glucitol was



3,4



25,26

Fig. 1. Fragmentation patterns of **3**, **4**, **25**, and **26**.

proved. Thus, the heptosyl residue occurring in the outer-core region of the LPS is linked to position 6 of the terminal glucopyranose residue.

EXPERIMENTAL

General methods.—The ^{1}H- and ^{13}C-NMR spectra were recorded with Varian Gemini 200 and Bruker AM 500 spectrometers on solutions in $CDCl_3$ (internal Me_4Si), and with a Bruker AM 360 L spectrometer on solutions of **3** and **4** in C_6D_6, as were the $^{1}H,^{1}H$ and $^{1}H,^{13}C$ chemical-shift-correlated (COSY) 2D spectra. Optical rotations were measured with a Jasco DIP-360 automatic polarimeter.

TABLE III

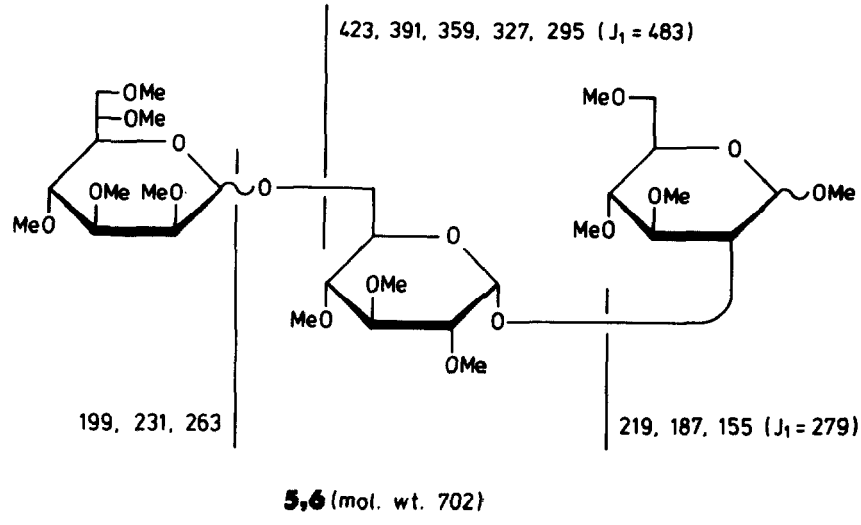
EIMS data of **5**, **6**, **27**, and **28**

Compound	Mol wt	*m/z* (% of base peak)
5	702	59 (18.8), 71 (39.2), 73 (15.7), 75 (49.1), 88 (68.0), 89 (37.8), 101 (100.0), 111 (22.4), 155 (11.0), 187 (19.1), 199 (24.0), 219 (13.6), 231 (5.3), 263 (13.0), 279 (8.0), 295 (0.6), 327 (1.8), 359 (4.6), 391 (1.5), 423 (2.3), 483 (6.1)
6	702	59 (16.0), 71 (38.4), 73 (13.6), 75 (50.2), 88 (84.5), 89 (32.1), 101 (100.0), 111 (20.9), 155 (10.9), 187 (22.5), 199 (18.5), 219 (15.3), 231 (4.4), 263 (10.7), 279 (7.4), 295 (1.0), 327 (1.7), 359 (4.6), 391 (2.7), 423 (2.7), 483 (5.9)
27	719	59 (24.2), 71 (30.3), 73 (13.0), 75 (33.6), 88 (72.2), 89 (45.8), 101 (100.0), 111 (17.5), 113 (3.5), 133 (6.8), 140 (3.0), 145 (14.5), 172 (9.4), 177 (1.2), 199 (37.2), 204 (0.8), 231 (10.0), 236 (80.8), 263 (21.2), 296 (1.9), 376 (0.5), 408 (0.9), 440 (0.6), 500 (3.4)
28	719	59 (22.4), 71 (30.2), 73 (11.5), 75 (39.2), 88 (79.4), 89 (47.4), 101 (100.0), 111 (16.4), 113 (4.2), 133 (7.7), 140 (3.4), 145 (12.8), 172 (9.2), 199 (25.1), 204 (0.8), 231 (8.1), 236 (65.6), 263 (20.4), 296 (0.5), 376 (0.2), 500 (1.7)

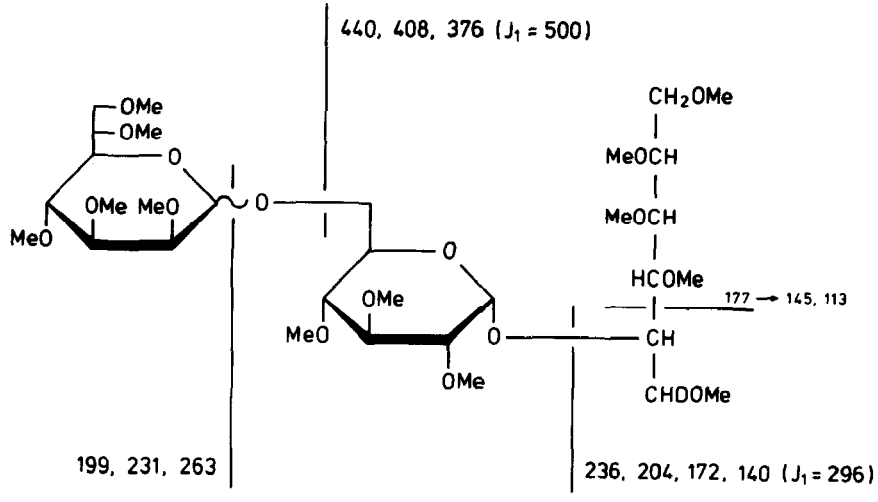
TLC was performed on silica gel (Merck, 5554), and column chromatography on silica gel (230–400 mesh, Merck). HPLC was performed on a column of Lichrosorb RB Si60 at 10 MPa, using a Siemens S100 chromatograph. GLC (temperature programme: 180° for 5 min, then 5°/min → 300°) and GLC–MS were performed as described [5]. All retention times were relative to that of maltitol nona-acetate and are listed in Table I.

*3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-benzyloxyethylidene)-α-*D*-glucopyranose* (**14**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (8.3 g, 20.2 mmol) in nitromethane (25 mL) were added 2,4,6-collidine (5.3 mL, 40.4 mmol), benzyl alcohol (10.5 mL, 101 mmol), and tetrabutylammonium bromide (6.45 g, 4.2 mmol). The mixture was stored at room temperature for 24 h, then diluted with $CHCl_3$ (300 mL), washed with water (3 × 100 mL), dried, and concentrated. Column chromatography (benzene, then 4:1 benzene–EtOAc) of the residue gave **13** (7.26 g, 82%), isolated as a syrup slightly contaminated with benzyl alcohol. ^{1}H-NMR data: δ 5.69 (d, 1 H, $J_{1,2}$ 5.3 Hz, H-1), 4.32 (ddd, 1 H, $J_{2,3}$ 3.0, $J_{2,4}$ 1.0 Hz, H-2), 5.21 (t, 1 H, $J_{3,4}$ 2.6 Hz, H-3), 4.91 (ddd, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.96 (m, 1 H, H-5), 4.20 (m, 2 H, H-6a,6b), 4.55 (s, 2 H, PhCH_2O), 2.10 (s, 9 H, 3 AcO), 1.79 (s, 3 H, CH_3).

A solution of **13** (7 g) in benzyl chloride (50 mL) was stirred at 100–130° with pulverised NaOH (10 g) for 4 h, then cooled, diluted with ether (250 mL), washed with water (3 × 50 mL), dried, filtered, and concentrated. Column chromatography (benzene, then 9:1 benzene–EtOAc) of the residue gave **14** (5.78 g, 62%), mp 68–71°, $[\alpha]_D^{20}$ +22° (*c* 0.8, $CHCl_3$). ^{1}H-NMR data: δ 5.76 (d, 1 H, $J_{1,2}$ 5.4 Hz, H-1), 4.40 (m, 1 H, H-2), 3.88 (dd, 1 H, $J_{3,2}$ 3.5, $J_{3,4}$ 3.8 Hz, H-3), 3.32–3.84 (m, 4 H, H-4,5,6a,6b), 1.74 (s, 3 H, CH_3).



5,6 (mol. wt. 702)



27,28 (mol. wt. 719)

Fig. 2. Fragmentation patterns of **5**, **6**, **27**, and **28**.

Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.21; H, 6.57. Found: C, 74.22; H, 6.54.

Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (**15**).—To a solution of **14** (5.13 g, 8.8 mmol) in nitromethane (50 mL) was added benzyl alcohol, and nitromethane (~5 mL) was then evaporated. Murcuric bromide (63.4 mg, 0.18 mmol) was added, and the mixture was stirred at 80° for 2 h, then cooled, diluted with $CHCl_3$ (200 mL), washed with water (3 × 50 mL), dried, and concentrated.

TABLE IV

^{1}H-NMR data (360 MHz, C_6D_6, internal Me_4Si) for **3** and **4**

Assignment	**3**				**4**			
	δ (ppm)		J (Hz)		δ (ppm)		J (Hz)	
	α	β	α	β	α	β	α	β
H-1	6.69	5.85	3.5	8.1	6.44	5.72	3.4	7.5
H-2	4.04	4.05	10.6	9.6	3.88	4.04	10.0	9.6
H-3	5.97	5.51	9.9	9.8	5.82	5.35	9.6	9.2
H-4	5.30	5.11	9.8	10.0	5.46	5.28	9.7	9.5
H-5	4.24–4.37 [a]	3.42–3.48 [a]			4.12–4.19 [a]	3.26–3.36 [a]		
H-6a	4.33	4.18	11.6	12.7	4.43	4.21–4.29 [a]	12.8	
H-6b	4.21	4.07	1.5	2.2	4.43 [b]	4.21–4.29 [a]		
H-1′	5.97	5.29	3.8	3.8	5.72	5.08	3.2	3.4
H-2′	5.05	5.20	10.5	10.4	4.94	5.15	10.4	10.1
H-3′	5.82	5.72	10.2	9.9	5.85	5.71	9.5	10.0
H-4′	5.04	5.14	10.5	10.2	5.21	5.21	9.9	9.9
H-5′	4.24–4.37 [a]	4.39		6.7	4.21–4.29 [a]	4.21–4.29 [a]		
H-6′a	3.55	3.62	10.7	10.9	3.47	3.56	10.8	10.6
H-6′b	3.46	3.49	2.7	2.3	3.93	3.96	3.1	3.3
H-1″	4.69	4.79 [b]	1.3		4.13	4.16	1.9	1.3
H-2″	5.60	5.60	3.2	3.2	5.70–5.76 [a]	5.70–5.76 [a]		
H-3″	5.66	5.67	10.2	10.3	5.16	5.17	10.3	10.3
H-4″	5.89	5.86	10.3	10.1	5.63	5.65	10.3	10.1
H-5″	4.80	4.58	2.2	2.4	3.33	3.29	2.4	2.5
H-6″	5.79 [b]	5.79 [b]			5.60	5.58	4.7	4.6
H-7″a	4.92	4.81	12.0	12.1	4.58	4.58	11.5	11.6
H-7″b	4.24–4.37 [a]	4.24–4.37 [a]			4.31	4.28	8.0	8.5

[a] Non-resolved high-order multiplet. [b] Non-resolved.

Column chromatography (benzene, then 95:5 benzene–EtOAc) of the residue gave **15** (4.46 g, 87%), mp 68–70°, $[\alpha]_D^{25}$ −24° (*c* 0.8, $CHCl_3$); lit. [7] $[\alpha]_D^{20}$ −16° ($CHCl_3$). ^{1}H-NMR data: inter alia, δ 5.08 (dd, 1 H, $J_{2,1}$ 8.0, $J_{2,3}$ 9.1 Hz, H-2), 4.42 (d, 1 H, H-1), 3.58–3.78 (m, 4 H, H-3,4,6a,6b), 3.48 (m, 1 H, H-5), 1.93 (s, 3 H, AcO).

Anal. Calcd for $C_{36}H_{38}O_7$: C, 74.21; H, 6.57. Found: C, 74.35; H, 6.54.

*Benzyl 3,4,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**16**).—Conventional *O*-deacetylation of **15** (4.1 g, 7 mmol) with 0.1 M sodium methoxide in $CHCl_3$–MeOH (4:1, 25 mL) and column chromatography (benzene–EtOAc, 4:1) of the product gave **16** (3.50 g, 92%), mp 70.5–72.5° (from ether–hexane), $[\alpha]_D^{25}$ −26.5° (*c* 0.7, $CHCl_3$); lit.[7] mp 70–71°, $[\alpha]_D^{20}$ −22.8° ($CHCl_3$); lit. [8] mp 87–88°, $[\alpha]_D$ −25° ($CHCl_3$); lit. [9] mp 89–90°, $[\alpha]_D$ −23.3°. ^{1}H-NMR data: inter alia, δ 4.36 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1), 3.76 (q, 1 H, $J_{6a,5}$ 2.1, $J_{6a,6b}$ 10.8 Hz, H-6a), 3.72 (q, 1 H, $J_{6b,5}$ 4.6 Hz, H-6b), 3.56–3.65 (m, 3 H, H-2,3,4), 3.48 (dq, 1 H, $J_{4,5}$ 9.4 Hz, H-5).

Anal. Calcd for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.41; H, 6.79.

*Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**12**).—To a solution of **18** [10] (2.41 g, 4.5 mmol) in CH_2Cl_2–EtOAc (9:1, 30 mL) at 0° was added a solution of $TiBr_4$ (3.3 g, 9 mmol) in CH_2Cl_2 (30

TABLE V

^{13}C-NMR data [a] (in ppm), 90.6 MHz, C_6D_6, internal Me_4Si) of the anomers of **3** and **4**

Assignment	**3** [b]		**4** [b]	
	α	β	α	β
C-1	88.72	93.88	88.72	93.76
C-2	74.72	75.27	73.89	74.21
C-3	70.55	72.16	71.32 [c]	73.64
C-4	69.62	69.10	68.74	68.52
C-5	70.76	73.32	70.55	72.90
C-6	62.98	62.28	62.12	61.40
C-1′	95.04	95.44	94.63	95.30
C-2′	69.58	70.01 [c]	71.64	70.40
C-3′	70.01 [c]	70.31 [c]	69.54	69.96 [c]
C-4′	70.88	69.71	69.96 [c]	69.96 [c]
C-5′	69.33	69.38	68.96	69.34
C-6′	66.77	67.33	69.68	69.84
C-1″	97.08	97.81	100.00	100.14
C-2″	70.01 [c]	69.62 [c]	68.90 [c]	68.90 [c]
C-3″	70.31 [c]	70.01 [c]	71.32 [c]	71.32 [c]
C-4″	64.74	64.79	64.94	65.05
C-5″	69.91	70.31 [c]	73.15	73.07
C-6″	67.95	67.89	67.31	67.25
C-7″	64.43	64.20	62.88	62.69

[a] Other signals: $COCH_3$ 168–171 ppm, $COCH_3$ 20–21 ppm. [b] Assignment by ^{1}H–^{13}C-COSY-NMR. [c] Non-resolved signal.

mL). The mixture was allowed to attain room temperature and, after 75 min, diluted with toluene (65 mL) and acetonitrile (25 mL); NaOAc (15 g) was then added. The suspension was shaken, filtered through a Celite pad, washed with aq $NaHCO_3$ and water, dried, and concentrated to give the bromide **19** [R_F 0.55 (hexane–EtOAc, 2:1)] that was used immediately.

To a solution of **16** (1.62 g, 3 mmol) in nitromethane (20 mL) and toluene (10 mL) were added mercuric cyanide (1.51 g, 6 mmol) and molecular sieves 4A (3 g), and the suspension was stirred under Ar. After 1 h, a solution of **19** in toluene (10 mL) was added, and the mixture was stirred at room temperature for 20 h, then diluted with $CHCl_3$ (200 mL), washed with aq 10% KI (3 × 50 mL), and concentrated. Column chromatography (benzene, then 4:1 benzene–EtOAc) of the residue gave the product (1.6 g) contaminated with **16**. A portion of this material was rechromatographed to yield pure benzyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (**11**), $[\alpha]_D^{20}$ +34.5° (*c* 2.4, $CHCl_3$). ^{13}C-NMR data: δ 102.77 (C-1), 95.04 (C-1′), 83.11 (C-3), 81.67 (C-2), 78.93, 78.84, 77.21, 77.13, 75.51, 75.07 (C-2′,3′, 4,4′, 5,5′), 68.65 (C-6), 62.69 (C-6′).

Anal. Calcd for $C_{63}H_{66}O_{12}$: C, 74.54; H, 6.55. Found: C, 74.50; H, 6.44.

Conventional *O*-deacetylation of **11** with 0.1 M sodium methoxide in $CHCl_3$–MeOH (1:4, 30 mL) and column chromatography (benzene, then 7:3 benzene–EtOAc) of the product gave **12** (1.25 g, 43%). Crystallisation from hexane–ether

gave **12**, mp 111–112°, $[\alpha]_D^{25}$ +41° (*c* 0.55, $CHCl_3$). ^{13}C-NMR data: δ 102.75 (C-1), 95.03 (C-1'), 83.20 (C-3), 81.55 (C-2), 79.03, 78.74, 77.28, 75.32, 74.98, 70.40 (C-2', 3', 4,4', 5,5'), 68.59 (C-6), 61.29 (C-6').

Anal. Calcd for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 75.20; H, 6.59.

*Benzyl 2-*O*-acetyl-3,5,6-tri-*O*-benzyl-β-*D*-glucofuranoside* (**20**).—A solution of 1,2-di-*O*-acetyl-3,5,6-tri-*O*-benzyl-α,β-D-glucofuranose [12] (1.88 g, 3.5 mmol) in CH_2Cl_2–benzyl alcohol (0.36 mL, 3.5 mmol) was stirred with molecular sieves 4A at room temperature for 1 h, then cooled to −10°, and trimethylsilyl triflate (0.7 mL, 3.5 mmol) was added. The mixture was allowed to attain room temperature and stirred for 2 h. Pyridine (0.2 mL) was added, and the mixture was diluted with $CHCl_3$ (100 mL), washed with water, dried, and concentrated. Column chromatography (light petroleum–EtOAc, 96:4) of the residue gave **20** (890 mg, 44%), $[\alpha]_D^{25}$ −69.5° (*c* 1.4, $CHCl_3$). ^{1}H-NMR data: inter alia, δ 5.27 (s, 1 H, H-2), 5.13 (s, 1 H, H-1), 4.35 (q, 1 H, $J_{3,4}$ 4.5, $J_{4,5}$ 9.2 Hz, H-4), 4.09 (o, 1 H, $J_{5,6a}$ 2.0, $J_{5,6b}$ 5.0 Hz, H-5), 4.07 (d, 1 H, H-3), 3.85 (q, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.68 (q, 1 H, H-6b), 2.06 (s, 3 H, AcO).

Conventional *O*-deacylation of **20** gave benzyl 3,5,6-tri-*O*-benzyl-β-D-glucofuranoside (**21**, 91%), $[\alpha]_D^{21}$ −63° (*c* 1.1, $CHCl_3$). ^{1}H-NMR data: inter alia, δ 4.99 (s, 1 H, H-1), 4.43 (q, 1 H, $J_{4,3}$ 4.9, $J_{4,5}$ 8.9 Hz, H-4), 4.28 (bs, 1 H, H-2), 4.07 (o, 1 H, $J_{5,6a}$ 2.0, $J_{5,6b}$ 5.3 Hz, H-5), 3.99 (q, 1 H, $J_{3,2}$ 1.3 Hz, H-3), 3.86 (q, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6a), 3.69 (q, 1 H, H-6b).

Anal. Calcd for $C_{34}H_{36}O_6$: C, 75.53; H, 6.71. Found: C, 75.28; H, 6.86.

*Benzyl 3,5,6-tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-glucofuranoside* (**23**).—Condensation of **21** (240 mg) with **19** (prepared from 250 mg of **18**) was performed as described for the preparation of **11**. Column chromatography (benzene, then 4:1 benzene–EtOAc) of the product yielded **22** (130 mg, 53%). ^{13}C-NMR data: inter alia, δ 106.00 (C-1), 96.00 (C-1'), 83.31, 81.35, 81.31, 80.19, 79.57, 77.15, 76.44 (C-2,2', 3,3', 4,4', 5'), 69.39 (C-6), 69.29 (C-5), 63.02 (C-6').

O-Deacetylation (0.1 M sodium methoxide in 1:4 $CHCl_3$–MeOH, 2 h) of **22** gave **23** (94%), $[\alpha]_D$ +12° (*c* 1.8, $CHCl_3$). ^{13}C-NMR data: inter alia, δ 106.11 (C-1), 96.69 (C-1'), 83.39, 81.38, 81.36, 80.18, 79.76, 77.08, 76.48 (C-2,2', 3,3', 4,4', 5'), 71.55 (C-5), 69.57 (C-6), 61.55 (C-6').

Anal. Calcd for $C_{61}H_{64}O_{11}$: C, 75.29; H, 6.63. Found: C, 74.94; H, 6.87.

*2-*O*-[6-*O*-(L-glycero-α- and -β-D-manno-Heptopyranosyl)-α-D-glucopyranosyl]-α,β-D-glucopyranose* (**1** *and* **2**).—To a solution of **12** (235 mg, 0.24 mmol) and 7-*O*-allyl-2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranosyl trichloroacetimidate [6] (**24**; 165 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) were added molecular sieves 4A (500 mg) under Ar. The mixture was stirred for 1 h, toluene-*p*-sulfonic acid (50 mg) was added, and stirring at room temperature was continued for 24 h. Saturated aq $NaHCO_3$ (6 mL) was added, the aqueous layer was extracted with CH_2Cl_2, and the combined organic solutions were dried and concentrated. HPLC (toluene–EtOAc, 94:6) of the residue gave, first, **7** (90 mg, 23.6%), $[\alpha]_D^{22}$ +46° (*c* 4.7, ether). ^{13}C-NMR data: inter alia, δ 102.84 (C-1), 98.32 (C-1''), 94.92 (C-1'),

83.32, 81.63, 79.62, 79.14, 78.84, 77.36, 75.33, 75.24, 75.12, 74.25, 73.88, 72.19, 69.95 (C-2,2′,2″/5,5′,5″ and C-6″), 70.88 (C-6′), 68.69 (C-7″), 65.40 (C-6).

Eluted second was **8** (117 mg, 34.2%), $[\alpha]_D^{25}$ +8° (*c* 6, ether). ^{13}C-NMR data: inter alia, δ 102.86 (C-1), 102.44 (C-1″), 94.60 (C-1′), 83.51, 82.76, 81.58, 79.92, 78.92, 78.06, 77.23, 75.48, 75.27, 75.07, 74.12, 73.19 (C-2,2′,2″/5,5′,5″ and C-6″), 70.84 (C-6′), 68.84, 68.72 (C-6,7″).

To a solution of **7** (90 mg) in oxolane (0.3 mL), MeOH (3 mL), and water (0.1 mL) were added 10% Pd–C (100 mg) and a few crystals of toluene-*p*-sulfonic acid. The suspension was stirred at room temperature for 66 h, neutralised with methanolic ammonia, filtered, and concentrated. HPLC (toluene–EtOAc, 9:1) of the residue yielded the deallylated product **9** (45 mg, 52%). ^{13}C-NMR data: inter alia, δ 102.83 (C-1), 98.23 (C-1″), 95.05 (C-1′).

To a solution of **9** in EtOH (5 mL) was added 10% Pd–C (100 mg), and the suspension was shaken under H_2 for 44 h, then filtered, and concentrated to yield **1** (15.3 mg, 99.2%). ^{13}C-NMR data: δ 101.46 (C-1″β), 101.42 (C-1″α), 99.84 (C-1β), 98.43 (C-1′α), 98.16 (C-1′β), 91.41 (C-1α), 80.81, 78.15, 77.72, 76.32, 75.10, 75.06, 73.34, 73.31, 73.08, 72.85, 72.10, 71.97, 71.87, 71.77, 71.60, 71.38, 71.27, 71.22, 70.82, 67.98 (C-2,2′,2″/5,5′,5″ and C-6″α,β), 66.78 (C-6′α), 66.71 (C-6′β), 65.07 (C-7″α,β), 62.73 (C-6β), 62.51 (C-6α).

Using the above procedure, **8** (105 mg) was converted into the deallylated product **10** (65 mg, 64%). ^{13}C-NMR data: inter alia, δ 102.79 (C-1), 102.34 (C-1″), 94.69 (C-1′).

Hydrogenation of **10** yielded **2** (22.1 mg, 99.2%). ^{13}C-NMR data: δ 102.05 (C-1″α,β), 99.29 (C-1β), 97.94 (C-1′α), 97.75 (C-1′β), 91.02 (C-1α), 80.93, 80.47, 77.79, 77.31, 76.18, 75.95, 74.77, 74.68, 74.31, 73.33, 73.19, 73.03, 72.96, 72.90, 72.85, 72.76, 72.58, 72.53, 72.42, 71.92, 71.29, 71.12, 71.02, 70.46, 67.56 (C-2,2′, 2″/5,5′,5″ and C-6″α,β), 69.73 (C-6′α), 69.42 (C-6′β), 64.34 (C-7″β), 64.02 (C-7″α), 62.34 (C-6β), 62.12 (C-6α).

Samples of **1** (12 mg) and **2** (15 mg) were acetylated [5]. Column (18 × 1 cm) chromatography of the products on Silica Gel 60 (Merck) with toluene–EtOH (5:1) yielded the dodeca-acetates **3** (23.2 mg), $[\alpha]_D^{16}$ +80° (*c* 0.7, $CHCl_3$), and **4** (11.6 mg), $[\alpha]_D^{16}$ +35° (*c* 0.7, $CHCl_3$). The ^{1}H- and ^{13}C-NMR data for **3** and **4** are shown in Tables IV and V, respectively.

Anal. Calcd for $C_{43}H_{58}O_{29}$: C, 49.71; H, 5.63. Found: for **3**, C, 49.38; H, 5.58; for **4**, C, 49.76; H, 5.59.

Reduction of **1** and **2** was done conventionally with NaB^2H_4. Acetylation [5] afforded **25** and **26**; methylation [14] and purification [15] yielded **27** and **28**. The methyl glycosides of **1** and **2** were obtained after treatment with methanolic M HCl for 16 h at 4°. Methylation [14] and purification [15] of the products gave **5** and **6**.

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